ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

VIROLOGY

Virology 316 (2003) 302–312

www.elsevier.com/locate/yviro

# Direct interaction of TFIIB and the IE protein of equine herpesvirus 1 is required for maximal *trans*-activation function

Randy A. Albrecht, Hyung K. Jang,[1] Seong K. Kim, and Dennis J. O'Callaghan*

*Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA 71130-3932, USA*

Received 15 July 2003; returned to author for revision 12 August 2003; accepted 18 August 2003

**Abstract**

Recently, we reported that the immediate-early (IE) protein of equine herpesvirus 1 (EHV-1) associates with transcription factor TFIIB [J. Virol. 75 (2001), 10219]. In the current study, the IE protein purified as a glutathione-*S*-transferase (GST) fusion protein was shown to interact directly with purified TFIIB in GST-pulldown assays. A panel of TFIIB mutants employed in protein-binding assays revealed that residues 125 to 174 within the first direct repeat of TFIIB mediate its interaction with the IE protein. This interaction is physiologically relevant as transient transfection assays demonstrated that (1) exogenous native TFIIB did not perturb IE protein function, and (2) ectopic expression of a TFIIB mutant that lacked the IE protein interactive domain significantly diminished the ability of the IE protein to *trans*-activate EHV-1 promoters. These results suggest that an interaction of the IE protein with TFIIB is an important aspect of the regulatory role of the IE protein in the *trans*-activation of EHV-1 promoters.


*Keywords:* EHV-1 gene regulation; IE protein, TFIIB

## Introduction

Equine herpesvirus type 1 (EHV-1) is employed as a model to study multiple aspects of herpesvirus infections, including viral gene expression, persistent infection mediated by defective interfering particles, and pathogenesis (Frampton et al., 2002; O'Callaghan and Osterrieder, 1999; Zhang et al., 2003). The EHV-1 genome is comprised of 78 genes that are coordinately regulated and temporally expressed as immediate-early (IE), early, late gamma-1, and late gamma-2 genes (Caughman et al., 1985; Gray et al., 1987a, 1987b), as is the case for other herpesviruses (Clements et al., 1977; Honess and Roizman, 1974; Roizman et al., 1975; Weinheimer and McKnight, 1987). The coordinated transcription of EHV-1 genes is regulated by six regulatory proteins that are expressed as one IE protein, four early proteins designated as EICP22, EICP27, EICP0, and IR2, and one late protein, ETIF (Bowles et al., 1997, 2000; Buczynski et al., 1999; Caughman et al., 1985, 1988; Derbigny et al., 2000, 2002; Gray et al., 1987a, 1987b; Grundy et al., 1989; Harty and O'Callaghan, 1991; Harty et al., 1990; Holden et al., 1995; Kim and O'Callaghan, 2001; Kim et al., 1997; Lewis et al., 1993, 1997; Smith et al., 1992, 1993, 1994, 1995; Zhao et al., 1992, 1995).

The IE protein (i) represses transcription from its own promoter (Smith et al., 1992), (ii) potently activates expression of early EHV-1 promoters (Smith et al., 1992, 1995), (iii) cooperates in a synergistic manner with the early EICP22 and EICP27 proteins to *trans*-activate early and some late promoters (Derbigny et al., 2000, 2002; Holden et al., 1995; Kim et al., 1997; Smith et al., 1993; Zhao et al., 1995), (iv) interacts physically with the EICP22 protein to enhance the DNA-binding ability of the IE protein (Derbigny et al., 2000, 2002; Kim et al., 1997), and (v) exhibits an antagonistic relationship with the early EICP0 protein, a powerful and promiscuous *trans*-activator of all classes of EHV-1 promoters (Bowles et al., 1997, 2000; Kim et al.,

* Corresponding author. Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130-3932. Fax: +1-318-675-5764.

*E-mail address:* docall@lsuhsc.edu (D.J. O'Callaghan).

[1] Present address: Department of Microbiology, College of Veterinary Medicine, Chonbuk National University, 664-14 Duckjin-dong, Jeonju 561-756, Korea.

0042-6822/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2003.08.017

1999, 2003). An acidic *trans*-activation domain (TAD) within the first 89 residues of the 1487 amino acid (aa) IE protein is necessary for its regulatory functions (Smith et al., 1994). In addition, aa 422 to 597 harbor a DNA-binding domain that specifically binds to the consensus DNA sequence 5′-ATCGT-3′ (Kim et al., 1995). Depending on the location of this consensus sequence relative to the transcription initiation site, the IE protein either *trans*-activates or represses transcription (Kim et al., 1995, 1999). Residues 963 to 970 contain an efficient nuclear localization signal, which is necessary for the activation properties of the IE protein (Smith et al., 1995).

The early IR2 protein is a truncated form of the IE protein that lacks the acidic TAD and the serine-rich tract, both of which are essential for IE protein function and virus replication (Garko-Buczynski et al., 1998; Kim et al., 2001; Harty and O'Callaghan, 1991; Smith et al., 1994). The observation that the IR2 protein retains the DNA-binding motif suggests that this protein functions as a negative regulator of some EHV-1 promoters, including the IE promoter (Kim et al., 1995; Smith et al., 1992).

General transcription factor TFIIB is a DNA-binding protein that functions in multiple stages of transcription (reviewed in Hampsey, 1998). The initial step in the formation of a preinitiation complex is the assembly of the TFIID–TFIIA (D–A) complex at the TATA box element (reviewed in Hampsey, 1998). TFIIB next localizes to the D–A complex via an interaction with TBP that is mediated by TFIIB aa 178 to 201 within the first direct repeat (Buratowski and Zhou, 1993; Ha et al., 1993; Yamashita et al., 1993) and stabilizes the interaction of the TATA box-binding protein (TBP) with the TATA box element (Gonzales-Couto et al., 1997). Following recruitment of TFIIB to the D–A complex, two TFIIB domains concomitantly engage sequences surrounding the TATA box element (Bagby et al., 1995; Nikolov et al., 1995). The TFIIB helix-turn-helix motif spanning $\alpha$-helices 4′ and 5′ (aa 270 to 290) binds to the TFIIB recognition element (BRE) that is immediately upstream of the TATA box element and contains the consensus sequence 5′-C/C-G/C-G/A-C-G-C-C-3′ (Lagrange et al., 1998). Basic helices BH2 and BH3 spanning aa 133 to 170 interact with sequences downstream of and adjacent to the TATA box element (Tsai et al., 2000). Localization of TFIIB to promoters results in the recruitment of the RNA polymerase II-TFIIF-TFIIH holoenzyme complex by a mechanism that involves binding of TFIIB residues encompassing the zinc ribbon (aa 15 to 37) to RNA polymerase II (Bangur et al., 1997, 1999; Bushnell et al., 1996; Hawkes and Roberts, 1999; Kim et al., 1994; Koleske and Young, 1994; Ossipow et al., 1995; Pardee et al., 1998; Pinto et al., 1994; Ranish et al., 1999; Thompson et al., 1993). The exact manner by which the RNA polymerase II holoenzyme complex is recruited to promoters remains unclear. The favored model, however, involves the recruitment of a preassembled holoenzyme complex that contains TFIIB to the D–A complex (Gonzalez-Couto et al., 1997; reviewed in Koleske and Young, 1995; Myer et al., 1998). TFIIB influences several aspects of transcription initiation, and various viral regulatory proteins physically interact with TFIIB in a manner that stimulates transcription of viral genes (Agostini et al., 1996; Benson et al., 1997; Haviv et al., 1998; Rank and Lambert, 1995; Tong et al., 1995; Veschambre et al., 1997; Yao et al., 1998).

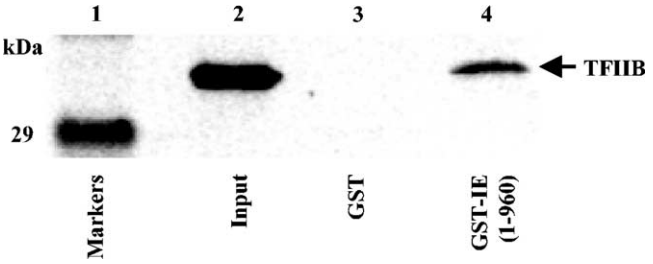


Fig. 1. Direct interaction of GST-IE (1–960) with TFIIB. Precipitation of TFIIB by GST-IE (1–960) in GST-pulldown assays was determined by SDS–PAGE and Western blot analysis. The input lane (Lane 2) refers to the amount of TFIIB included in each reaction. Western blot analysis with the anti-TFIIB polyclonal antibody demonstrated the presence of TFIIB within the precipitates (Lane 4). The sizes of the molecular weight markers are indicated to the left.

Recently, we showed that the EHV-1 IE protein associates with TFIIB; however, it was not shown whether the association of these two proteins resulted from a direct interaction or required other cellular factors (Jang et al., 2001). In this article, we employed purified IE and TFIIB proteins to demonstrate that this interaction is direct. In addition, a library of mutant forms of the TFIIB protein was utilized to identify the TFIIB domain that mediates its interaction with the IE protein. The physiological relevance of this interaction was shown by transient transfection assays that demonstrated that the deletion of the IE protein-binding domain within TFIIB greatly reduced (75%) the ability of the IE protein to *trans*-activate EHV-1 promoters, but did not have any inhibitory effect on VP16, a powerful *trans*-activator encoded by herpes simplex virus 1.

## Results

### *TFIIB directly interacts with GST-IEP (1–960)*

Previous results showed that the EHV-1 IE protein associates with TFIIB in protein-binding assays (Jang et al., 2001). To examine whether the IE protein directly interacts with TFIIB, purified [glutathione-*S*-transferase (GST)-free] TFIIB and GST-IE (1–960) were employed in GST-pulldown assays. Western blot analyses of the precipitates with an anti-TFIIB polyclonal antibody indicated that purified GST-IE (1–960) directly precipitated TFIIB (Fig. 1, Lane 4). GST by itself, the negative control, was unable to precipitate the purified TFIIB protein (Fig. 1, Lane 3). Also, the finding that the glutathione-Sepharose beads failed to pre-

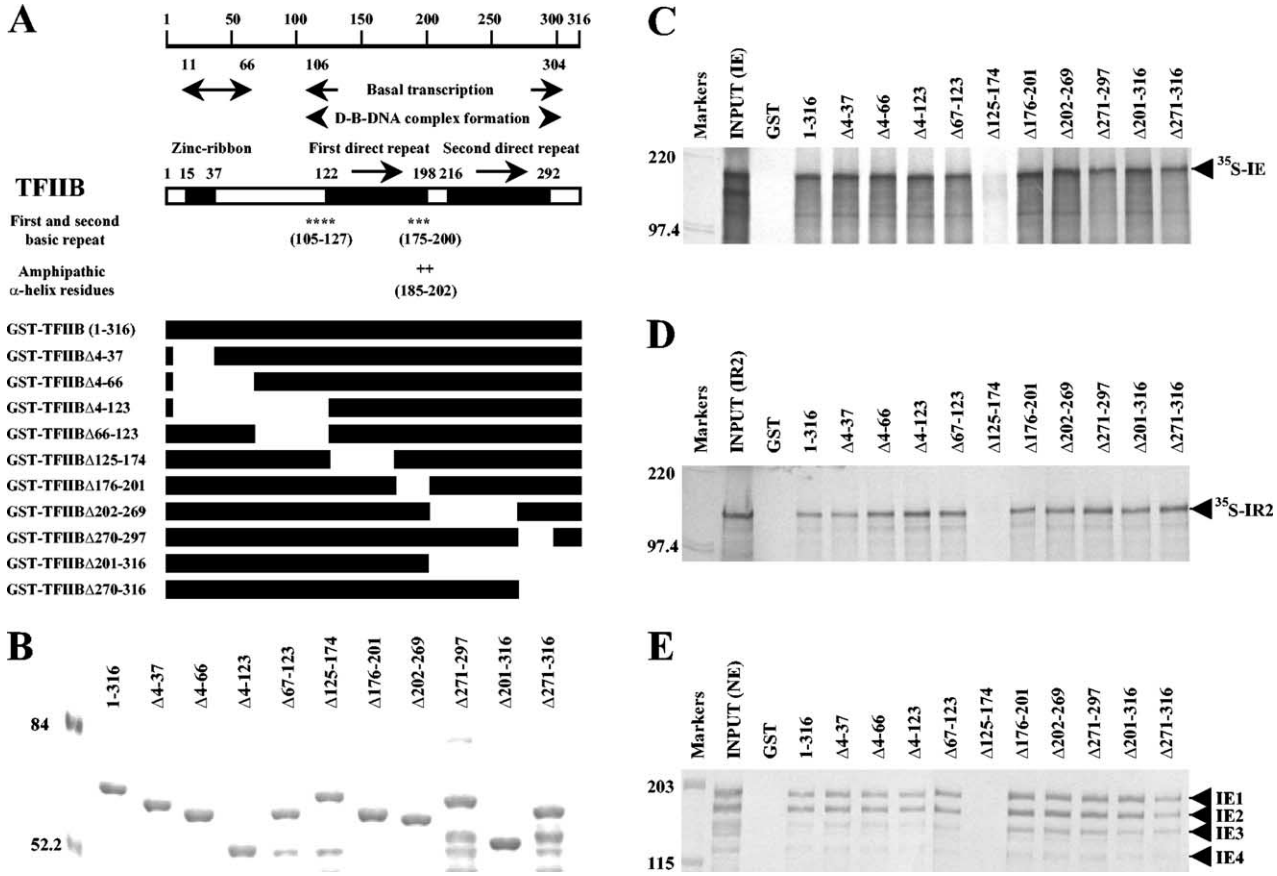


Fig. 2. Deletion mutants of the GST-TFIIB fusion protein employed to map the IE protein-binding domain within TFIIB. (A) Schematic representation of GST-TFIIB fusion proteins tested in protein-binding assays. The amino acid positions of the TFIIB functional domains are indicated at the top of the figure. The amino acid sequence that is deleted in each TFIIB mutant is indicated to the left. (B) Coomassie blue-stained polyacrylamide gel of the *E. coli* expressed GST-TFIIB fusion proteins. Molecular weight markers (kDa) are indicated on the left. (C) The GST-TFIIB proteins were assayed in GST-pulldown assays for interaction with $^{35}$S-IE protein. (D) Parallel GST-pulldown assays tested the ability of the GST-TFIIB proteins to precipitate the $^{35}$S-IR2 protein. (E) Interaction of TFIIB full-length and mutant GST-fusion proteins with the native IE protein from nuclear extracts of EHV-1-infected L-M cells. IE1 to IE4 represent a family of IE protein species that were characterized previously (Caughman et al., 1998). In C to E, input lanes represent the amount of IE or IR2 protein included in each reaction to assess the relative binding efficiency of each GST-TFIIB fusion protein.

cipitate purified TFIIB (Fig. 1, Lane 3) indicated that the GST moiety was absent from the recombinant TFIIB following its purification (see Materials and methods).

### *Identification of an IE protein-binding domain within TFIIB*

To map the IE protein-binding domain within TFIIB, a panel of GST-TFIIB deletion and truncation mutants was generated, purified, and employed in GST-pulldown assays (Figs. 2A and B). The results of the initial GST-pulldown assays involving [$^{35}$S]methionine-labeled IE protein or IR2 protein are presented in Figs. 2C and D, respectively. GST-TFIIB (1-316) interacted with both the in vitro transcribed/translated (IVTT) IE and the IR2 proteins. The deletion mutants GST-TFIIBΔ4–37, GST-TFIIBΔ4–66, GST-TFIIBΔ4–123, GST-TFIIBΔ67–123, GST-TFIIBΔ176–201, GST-TFIIBΔ202–269, GST-TFIIBΔ271–297, GST-TFIIBΔ201–316, and GST-TFIIBΔ271–316 precipitated both the IE and the IR2 proteins with relatively the same efficiency as did the full-length GST-TFIIB. However, GST-TFIIBΔ125–174 was not able to precipitate either of the IVTT-synthesized EHV-1 proteins, suggesting that aa 125 to 174 within the first direct repeat of TFIIB contain an IE protein-binding domain.

To confirm these mapping results, the ability of the panel of GST–TFIIB fusion proteins to react with native IE protein present in EHV-1-infected murine cells was examined. The binding reactions were performed with nuclear extracts prepared from L-M cells mock-infected or infected for 6 h with EHV-1 at an m.o.i. of 15 plaque forming units (PFU) per cell. Native IE protein within each precipitate was detected by Western blot analysis with an anti-IE polypeptide polyclonal antibody (Smith et al., 1994). As was the case for the IVTT-generated proteins, GST-TFIIB (1-316) interacted with each of the four native IE protein species (Caughman et al., 1988) from infected cell nuclear extracts, while GST-TFIIBΔ125–174 was incapable of precipitating any of the

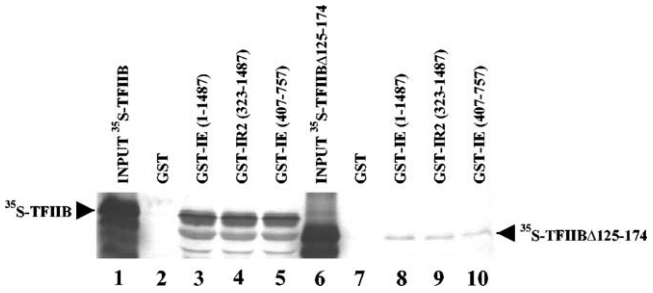


Fig. 3. TFIIB amino acids 125 to 174 physically interact with the IE protein in a GST-pulldown assay. Input lanes represent the amount of $^{35}$S-TFIIB or $^{35}$S-TFIIBΔ125–174 included in the reactions, which were employed to assess the relative binding efficiency of each GST fusion protein.

IE protein species (Fig. 2E). These results also indicate that aa 125 to 174 within the first direct repeat of TFIIB harbor an IE protein-binding domain. The inability of GST alone to precipitate the test proteins indicated the specificity of the GST-pulldown assays.

*TFIIB aa 125–174 mediate binding to the IE protein*

To confirm that aa 125 to 174 of TFIIB specifically interact with the IE and IR2 proteins, the ability of different forms of the IE protein produced as GST fusion proteins to precipitate [$^{35}$S]methionine-labeled TFIIB or TFIIBΔ125–174 was examined (Fig. 3). We previously reported that aa 407–757 of the IE protein are important for its association with TFIIB (Jang et al., 2001). Therefore, GST-IE (407–757) was included in these experiments to demonstrate that this sequence is sufficient to mediate a direct interaction with TFIIB. As shown in Fig. 3, GST-IE (1–1487), GST-IR2 (323–1487), and GST-IE (407–757) precipitated full-length, $^{35}$S-labeled TFIIB with equal efficiency, indicating that IE aa 407–757 participate in the direct interaction with TFIIB. $^{35}$S-TFIIBΔ125–174 that lacks the putative IE protein interactive domain failed to bind to any of these three GST-IE fusion proteins. These results are in agreement with the mapping data in Fig. 2 and indicate that the IE protein-binding domain maps to aa 125 to 174 within the first direct repeat of TFIIB.

To identify conclusively the IE protein-binding domain, additional GST-TFIIB mutants (Fig. 4A) were examined for an interaction with the $^{35}$S-IE protein. In this experiment, the two GST-TFIIB proteins that contain the IE protein-binding domain within aa 125 to 174 interacted efficiently with $^{35}$S-IE (Fig. 4B). However, the two TFIIB proteins that lack aa 125 to 174, GST-TFIIB (1–123) and GST-TFIIB (175–316), were incapable of precipitating the IE protein. Thus, the EHV-1 IE protein interacts with this cellular transcription factor, and a domain of the IE protein within aa 407 to 757 directly interacts with TFIIB residues 125 to 174.

*TFIIBΔ125–174 does not perturb the expression of the IE gene*

Before assessing the effect the mutant forms of TFIIB exert on IE gene function, it was important to show that ectopically expressed TFIIB species did not alter the synthesis of the IE protein. L-M cells were cotransfected with the pSVIE expression construct and either the TFIIB (1–316) or the TFIIBΔ125–174 expression construct. Cell lysates were prepared 48 h posttransfection, and Western blot analyses that employed IE polypeptide-specific antiserum were performed to determine whether the different forms of the TFIIB proteins influenced the steady-state levels of the IE protein (Fig. 5). The internal control was a cellular protein that was nonspecifically detected by the anti-IE polypeptide antibody (Jang et al., 2001) and indicated that each sample contained an equal amount of protein. Compared to the steady-state level of the IE protein in cells transfected with just the pSVIE expression construct (Fig. 5, Lane 3), neither the expression of TFIIB (1–316) nor TFIIBΔ125–174 adversely affected IE protein synthesis (Fig. 5, Lanes 5 and 6, respectively), indicating that the mutant form of TFIIB does not down-regulate the constitutively active SV40 promoter or adversely affect IE protein

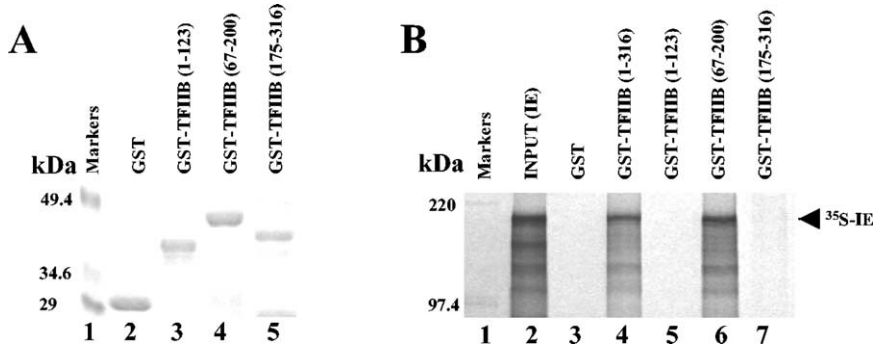


Fig. 4. Protein–protein interaction assays confirmed that amino acids 125 to 174 of TFIIB harbor the minimal IE protein-binding domain. (A): Coomassie blue stained polyacrylamide gel of the *E. coli* expressed GST-TFIIB fusion proteins. (B): GST-pulldown assay assessed the ability of the GST fusion proteins to interact with the $^{35}$S-IE protein. The input lane represents the amount of $^{35}$S-IE protein included in each reaction and was employed to assess the relative binding efficiency of each GST fusion protein. In both panels, molecular weight markers (kDa) are indicated on the left.

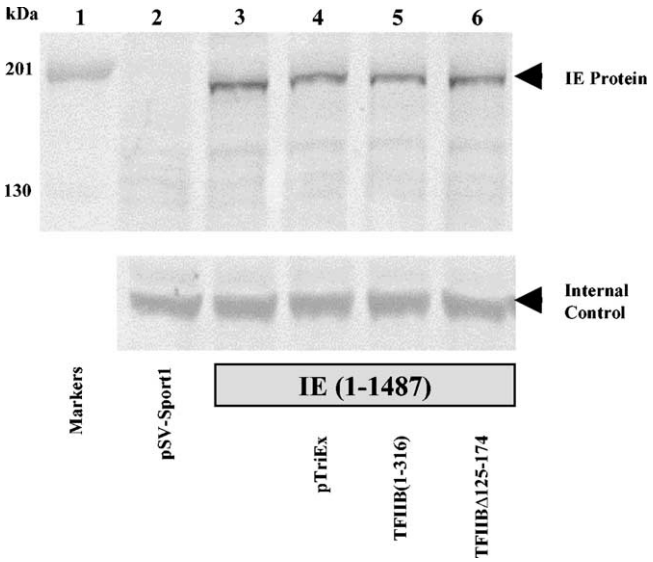


Fig. 5. The steady-state level of the IE protein was not reduced by overexpression of TFIIBΔ125–174. Murine fibroblast L-M cells were transfected with 0.3 pmol of the pSVIE expression plasmid in the absence or presence of 0.4 pmol of either the parental cloning vector (pTriEx-1) (lane 4) or the plasmids expressing native TFIIB (1–316) (Lane 5) or TFIIBΔ125–174 (Lane 6). As a negative control, the cells were transfected with the parental expression vector pSV-Sport1 (Lane 2), which was employed to clone the IE gene. Western blot analyses with an anti-IE polypeptide-specific antibody were performed as described under Materials and methods.

stability. As a negative control for the specificity of the anti-IE polypeptide antibody, cell lysates were prepared from cells transfected with the empty pSV-Sport 1 expression construct (Fig. 5, Lane 2). Additionally, cotransfection of the empty pTriEx-1 vector with the pSVIE construct also did not interfere with the synthesis of the IE protein (Fig. 5, Lane 4).

### *Importance of the interaction between the IE protein and TFIIB*

To ascertain whether the interaction between TFIIB and the IE protein is essential for full activity of the IE protein as a *trans*-activator, transient transfection assays were carried out in murine fibroblast cells. As shown in Fig. 6, the IE protein significantly activated the EHV-1 early EICP27 promoter greater than four-fold over basal levels observed for the EICP27 promoter alone (Fig. 6, Bar 2), which is consistent with our previously published findings (Bowles et al., 1997, 2000; Kim et al., 1997, 1999; Smith et al., 1992; Zhao et al., 1995). Expression of native TFIIB from transfected pTriExTFIIB (1–316) increased the level of chloramphenicol acetyl transferase (CAT) activity greater than three-fold over the basal level (Fig. 6, Bar 3). This observation suggests that although the level of endogenous TFIIB was sufficient for promoter activity, the amount of endogenous TFIIB was limiting for maximal transcription from the EICP27 promoter. Increasing amounts of native TFIIB significantly increased the ability of the IE protein to *trans*-activate the EICP27 promoter ($P < 0.005$; Fig. 6, Bars 4 to 6). The expression of TFIIBΔ125–174 enhanced the basal expression of the EICP27 promoter (Fig. 6, Bar 7). In marked contrast, the regulatory function of the IE protein was proportionately decreased in the presence of increasing molar concentrations of plasmid TriExTFIIBΔ125–174 that expresses TFIIB lacking the IE protein interactive domain (Fig. 6, Bars 8 to 10). The *trans*-activation function of the IE protein was decreased by more than 65% when 0.4 pmol of pTriExTFIIBΔ125–174 was cotransfected with the IE expression construct (Fig. 6, Bar 10, $P < 0.005$). Similar results were observed with the early viral thymidine kinase (TK) promoter-CAT reporter construct (data not shown), in that expression of TFIIB lacking the IE protein interactive domain significantly reduced *trans*-activation of this EHV-1 promoter by 70%. These results suggested that the interac-

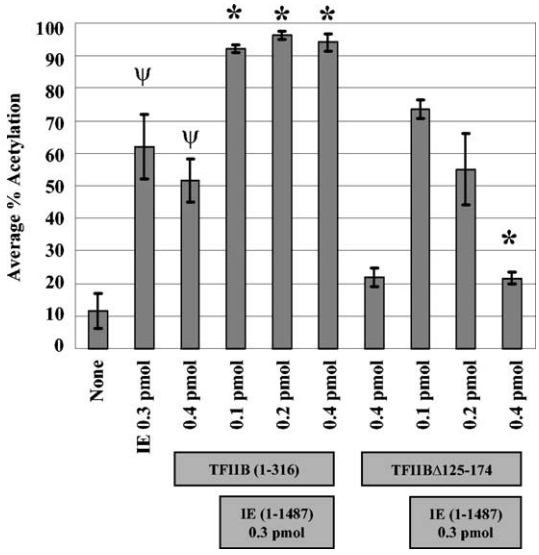


Fig. 6. Deletion of the IE protein-binding domain within TFIIB reduces the ability of the IE protein to *trans*-activate the EHV-1 early EICP27 promoter. Transient transfection assays employing the EICP27-CAT reporter were performed to test the effect of ectopically expressed full-length TFIIB or TFIIBΔ125–174 on the regulatory activity of the IE protein. L-M cells were transfected with 1.4 pmol of the pEICP27-CAT reporter (pEICP27-CAT) alone (Bar 1) or were cotransfected with pEICP27-CAT and one of the effector constructs (Bars 2 to 10). pSVIE was cotransfected at 0.3 pmol per transfection (Bars 2, 4, to 6, and 8 to 10). pSVIE was cotransfected with increasing molar concentrations of 0.1, 0.2, or 0.4 pmol of pTriExTFIIB (1–316) (Bars 4 to 6, respectively). pSVIE was cotransfected with increasing molar concentrations of 0.1, 0.2, or 0.4 pmol of pTriExTFIIBΔ125–174 (Bars 8 to 10, respectively). The data are representative of four independent experiments in which each sample was assayed independently in triplicate. The error bars show the standard deviations. * denotes statistical significance ($P < 0.005$) when the CAT activity of a sample was compared to the CAT activity achieved when cells were transfected with pEICP27-CAT and pSVIE. $\psi$ denotes statistical significance ($P < 0.005$) when the CAT activity of a sample was compared to the CAT activity achieved when cells were transfected with pEICP27-CAT in the absence of any vectors encoding effector proteins.

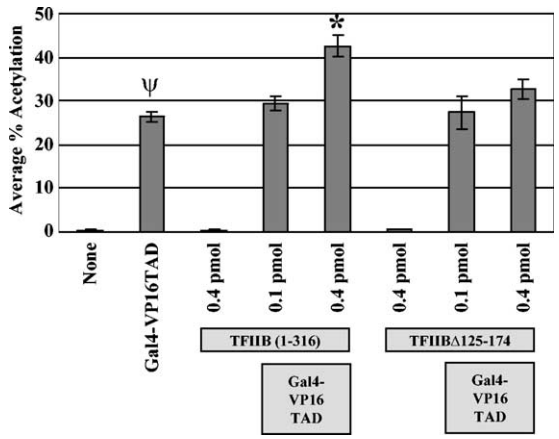


Fig. 7. Ectopic expression of a TFIIB mutant that lacks the IE protein interactive domain does not inhibit the *trans*-activation function of VP16. Gal4 two-plasmid assays employing the Gal4-responsive promoter pG5EC were performed to determine whether TFIIBΔ125–174 participated normally within preinitiation complexes directed by the VP16 TAD. In each sample, L-M cells were transfected with at least 0.5 pmol of the pG5EC reporter (Bars 1 to 8). pGal4-VP16TAD was transfected at 0.1 pmol per transfection (Bars 2 to 8). pTriExTFIIB (1–316) and pTriExTFIIBΔ125–174 were individually transfected at 0.4 pmol (Bars 3 and 6, respectively). pGal4-VP16TAD was cotransfected with increasing molar concentrations of 0.1 or 0.4 pmol of either pTriExTFIIB (1–316) (Bars 4 and 5) or pTriExTFIIBΔ125–174 (Bars 7 and 8). The data are representative of four independent experiments in which each sample was assayed independently in triplicate. The error bars show the standard deviations. * denotes statistical significance ($P < 0.005$) when the CAT activity of a sample was compared to the CAT activity achieved when cells were transfected with pG5EC and pGal4-VP16TAD. $\psi$ denotes statistical significance ($P < 0.005$) when the CAT activity of a sample was compared to the CAT activity achieved when cells were transfected with pG5EC in the absence of any vectors encoding effector proteins.

tion of TFIIB with the IE protein is important for the IE protein to maximally *trans*-activate EHV-1 promoters.

### *TFIIBΔ125–174 specifically attenuates trans-activation by the IE protein*

Overexpression of TFIIBΔ125–174 might not specifically perturb the regulatory function of the IE protein, but instead this TFIIB mutant may be intrinsically defective in participating in basal as well as activator-dependent transcription. This possibility was explored in the Gal4 two-plasmid transient transfection assay that included the 78 aa *trans*-activation domain of VP16 from herpes simplex virus type 1, synthesized as a Gal4 fusion protein (Gal4-VP16TAD; Smith et al., 1994). The VP16 TAD stimulates the formation of a preinitiation complex by multiple mechanisms that include a direct interaction with a region of TFIIB that encompasses its second basic repeat (aa 175–200; Chou and Struhl, 1997; Lin et al., 1991; Roberts et al., 1993). As presented in Fig. 7, Gal4-VP16TAD significantly stimulated transcription from the Gal4-responsive promoter as indicated by a significant increase in CAT reporter activity (Fig. 7, Bar 2, $P < 0.005$). Contrary to results obtained with the EHV-1 EICP27 promoter, ectopic expression of either TFIIB (1–316) or TFIIBΔ125–174 did not result in any activation of the G5EC promoter (Fig. 7, Bars 3 and 6, respectively). Increasing the amount of ectopically supplied TFIIB (1–316) potentiated *trans*-activation of the G5EC promoter by the Gal4-VP16TAD fusion protein (Fig. 7, Bars 4 and 5). Specifically, when 0.4 pmol of TFIIB (1–316) was employed (Fig. 7, Bar 5), VP16 *trans*-activation of the G5EC promoter was significantly increased ($P < 0.005$). In contrast to the negative effects of TFIIBΔ125–174 on the regulatory function of the IE protein (Fig. 6, Bar 10), cotransfection of the pGal4-VP16TAD expression vector with 0.4 pmol of the plasmid TriExTFIIBΔ125–174 did not have an inhibitory effect on VP16 function; indeed, a reproducible increase in VP16 *trans*-activation of the G5EC promoter was observed (Fig. 7, Bar 8). These data indicate that the TFIIBΔ125–174 mutant is specifically defective in participating in IE protein-mediated activation of EHV-1 promoters. The results of Figs. 6 and 7 reveal that ectopic expression of a mutant TFIIB protein that lacks sequences that mediate an interaction with the IE protein, but not with VP16, selectively fails to allow maximal *trans*-activation by the IE protein. Overall, these data indicate that the physical interaction of the IE protein with TFIIB plays an important role in viral gene expression.

## Discussion

Previously, we presented evidence that residues 407 to 757 within the IE protein's helix-loop-helix motif associate with TFIIB during viral infection (Jang et al., 2001). The EHV-1 IE protein *trans*-activates viral promoters by actively recruiting components of the RNA polymerase II preinitiation complex to promoters, a mechanism shared by other alphaherpesvirus regulatory proteins. HSV-1 VP16, an essential activator of immediate-early promoters, binds to multiple components of the preinitiation complex (Hall and Struhl, 2002), including direct interactions with TFIIB (Chou and Struhl, 1997; Lin et al., 1991; Roberts et al., 1993), TBP (Ingles et al., 1991; Stringer et al., 1990), $TAF_{II}32$ (Klemm et al., 1995), and TFIIA (Kobayashi et al., 1995). In addition, HSV-1 ICP4 stimulates transcription from late promoters via interactions with TFIID in a manner that is dependent on an additional interaction with $TAF_{II}250$ (Carrozza and DeLuca, 1996; Grondin, 2000; Gu and DeLuca, 1994).

In this article, we determined that there is a direct interaction between TFIIB and this major EHV-1 regulatory protein, and that aa 125 to 174 in TFIIB harbor the IE protein interactive domain. Our previous work with the yeast II hybrid system revealed that the IE protein interacts with itself to form dimers (Derbigny et al., 2000). This dimerization of the IE protein, possibly via its helix-loop-helix motif, exposes key hydrophobic residues that enable the IE protein to interact with TFIIB. The TFIIB residues

(aa 125 to 174) that associate with the IE protein are located within the first direct repeat of TFIIB. Residues 159 to 168 of the first direct repeat of TFIIB are rich in hydrophobic amino acids (Bagby et al., 1995). Thus, the interaction of these two proteins appears to be mediated by hydrophobic domains, which is consistent with our previous hypothesis (Jang et al., 2001). The mapping of the IE protein-binding domain within this core supports our suggested mechanism by which the IE protein interacts with both DNA and TFIIB (Jang et al., 2001). The demonstration that the IR2 protein binds to this TFIIB domain with an efficiency comparable to that of the IE protein raises an interesting possibility. Since the IR2 protein lacks the potent acidic *trans*-activation domain within aa 3 to 89 of the full-length IE protein, the IR2 protein may function as a negative regulator of viral gene expression by squelching the limited supply of transcription factors such as TFIIB.

Transient transfection assays provided two observations concerning the in vivo importance of the TFIIB-IE protein interaction with respect to EHV-1 promoter *trans*-activation. First, ectopically supplied native TFIIB enhanced the basal levels of transcription initiated from the EICP27 promoter (greater than a three-fold increase in the presence of 0.4 pmol of the native TFIIB expression construct) and augmented the regulatory function of the IE protein (approximately a 51% increase). Second, and a more significant observation, exogenously supplied mutant TFIIB lacking the domain that mediates its interaction with the IE protein selectively impaired the *trans*-activation function of the IE protein (approximately a 65% decrease in the presence of 0.4 pmol of the mutant TFIIBΔ125–174 expression construct). In these assays, TFIIBΔ125–174 increased the basal level of transcription from the EICP27 promoter by approximately 83% (Fig. 6, lane 7). This observation indicated that the mutant TFIIB was still capable of interacting with components of the preinitiation complex, resulting in the increased basal transcription from this viral promoter. We speculate that the impaired regulatory function of the IE protein in the presence of this mutant TFIIB results from competition of mutant TFIIB and wild-type TFIIB for binding to RNA polymerase II, which ultimately reduces the recruitment of RNA polymerase II to viral promoters. Furthermore, the ability of the TFIIB mutant to augment the regulatory function of the VP16 TAD suggests that this mutant protein retains activities that are necessary for it to function properly within the preinitiation complex. This observation supports our notion that the diminished regulatory function of the IE protein is a result of an absence of necessary protein–protein interactions between the IE protein and TFIIB, and not of improper function of the TFIIB mutant. These interpretations are supported by the revelation that overexpression of TFIIBΔ125–174 did not negatively influence the steady-state levels of the IE protein during the duration of the transient transfection assays.

The ability of TFIIBΔ125–174 to enhance gene expression in our assays is in agreement with other findings in which overexpressed TFIIBΔ148–163 increased reporter expression controlled by the human immunodeficiency virus type 1 long terminal repeat (LTR) (Veschambre et al., 1997). Collectively, these data suggest that TFIIBΔ125–174 is competent for activator-independent initiation of transcription from the heterologous promoters tested in these experiments. The failure of exogenously provided TFIIBΔ125–174 to block completely the *trans*-activation function of the IE protein could be explained by insufficient quantities of TFIIBΔ125–174 to outcompete the endogenous TFIIB, which was present in levels sufficient to allow formation of preinitiation complexes that support activated transcription of EHV-1 genes.

Overall, these data support our assumption that the interaction of the IE protein with general transcription factor TFIIB is necessary for the *trans*-activation properties of the IE protein (Jang et al., 2001). However, the mechanism(s) by which the acidic *trans*-activation domain (aa 3 to 89) influences transcription remains obscure. In addition, IE protein residues 1029 to 1487 appear to possess a domain(s) that enhances the regulatory function of the IE protein by an unknown mode of action (Buczynski et al., 1999; Smith et al., 1994). Future endeavors will involve ascertaining whether these domains within the IE protein interact with other transcription factors and/or RNA polymerase II in a fashion that facilitates the formation of preinitiation complexes at EHV-1 promoters and/or stimulates elongation of transcribing RNA polymerase II holoenzyme complexes.

## Materials and methods

### *Plasmid construction*

To facilitate the mapping of the domain of TFIIB that mediates its interaction with the IE protein, a panel of GST-TFIIB deletion mutants was constructed (Fig. 1A). Plasmid GSTKG-TFIIB, which contains the entire TFIIB coding sequence cloned in-frame with the GST gene and expresses the fusion protein GST-TFIIB (1–316), was described previously (Jang et al., 2001). Digesting pGBK-TFIIB with *Sma*I and *Sal*I, and ligating the released TFIIB gene to pGEX-4T1 (Promega, Madison, WI) that was digested with *Sma*I and *Sal*I, generated plasmid GST4T1-TFIIB. Plasmid GST-TFIIBΔ4–37 was generated by a two-step cloning strategy. First, pGSTKG-TFIIB was digested with *Acc*I and *Sal*I, followed by blunt-ending the 5′ end with Klenow enzyme, and inserting a *Cla*I linker d(pCCCATCGATGGG) (New England Biolabs, Beverly, MA) at codon 4 to generate pGSTKG-TFIIBaa4C. Plasmid GSTKG-TFIIBaa4C was subsequently digested with *Cla*I and *Hin*dIII and ligated with a PCR-amplified segment of TFIIB spanning codons 38 to 311. The forward primer was 5′-CCATCGATGGCTTGGTTGTAGGTGACCGGG-3′ and the reverse primer was 5′-CCCAAGCTTTTATAGCTGTGGTAGTTTGTC-3′. GST-TFIIBΔ4–66 is a derivative

of pGSTKG-TFIIBaa4C. Plasmid GSTKG-TFIIBaa4C was digested with *Cla*I and *Hin*dIII and ligated with a PCR-amplified segment of TFIIB spanning codons 67 to 311. The forward primer was 5′-CCATCGATGTTGGAGATTCT-CAGAATCCTC-3′ and the reverse primer was 5′-CCCAAGCTTTTATAGCTGTGGTAGTTTGTC-3′. Plasmid GST-TFIIBΔ4–123 is a subclone of pGSTKG-TFIIBaa4C and was generated by inserting a PCR-amplified segment of TFIIB corresponding to codons 124 to 311. The forward primer was 5′-CCATCGATATGGCAGACA-GAATCAATCTAC-3′ and the reverse primer was 5′-CCCAAGCTTTTATAGCTGTGGTAGTTTGTC-3′. Plasmid GST-TFIIBΔ67–123 was derived from pGST4T1-TFIIBaa67N, which was generated by partially digesting pGST4T1-TFIIB with *Xho*I, blunt-ending the 5′ end, and inserting the *Nco*I linker d(pCCCATGGG) (New England Biolabs) at codon 67. Plasmid GST4T1-TFIIBaa67N was subsequently digested with *Nco*I and religated to generate pGST-TFIIBΔ67–123. Plasmid GST-TFIIBΔ125–174 was derived from pGSTKG-TFIIBaa174N, which was generated by digesting pGSTKG-TFIIB with *Avr*II, blunt-ending the 5′ end, and inserting the *Nco*I linker d(pCAGCCATG-GCTG) (New England Biolabs). Plasmid GSTKG-TFIIBaa174N was subsequently digested with *Nco*I and religated to generate pGST-TFIIBΔ125–174. Plasmid GST-TFIIBΔ176–201 was generated by digesting pGSTKG-TFIIB with *Avr*II and *Eco*47III, blunt-ending the 5′ end with Klenow enzyme, and religating the resulting DNA fragment. Plasmid GST-TFIIBΔ202–269 was cloned by digesting pGSTKG-TFIIB with *Eco*47III and *Ppu*MI, blunt-ending the 5′ end, and religating the resulting DNA fragment. Plasmid GST-TFIIBΔ271–297 was derived from pGSTKG-TFIIBaa270B, which was cloned by digesting pGSTKG-TFIIB with *Ppu*MI, blunt-ending the 5′ end, and inserting the *Bgl*II linker d(pGGAAGATCTTCC) (New England Biolabs). Plasmid GSTKG-TFIIBaa270B was subsequently digested with *Bgl*II and religated to generate pGST-TFIIBΔ271–297. Plasmid GST-TFIIBΔ201–316 was cloned by digesting pGSTKG-TFIIB with *Eco*47III and *Hin*dIII, blunt-ending the 5′ end, and religating the resulting DNA fragment. Plasmid GST-TFIIBΔ271–316 was cloned by digesting pGSTKG-TFIIB with *Ppu*MI and *Hin*dIII, blunt-ending the 5′ end, and religating the resulting DNA fragment. pGST-TFIIB (1–123) was created by digesting pGSTKG-TFIIB with *Nco*I and *Hin*dIII, filling in the 5′ overhang with Klenow enzyme, and self-ligating the DNA fragment. pGST-TFIIB (67–200) was cloned by digesting pGST-TFIIBΔ4–66 with *Eco*47III and *Hin*dIII, and self-ligating the resulting Klenow-treated restriction enzyme fragment. pGST-TFIIB (175–316) was generated by cutting pGST-TFIIBΔ4–66 with *Cla*I and *Avr*II and self-ligating the resulting Klenow-treated restriction enzyme fragment. Plasmids pGST-IE, pGST-IR2 (323–1487), pGST-IE (1–960), and pGST-IE (407–757), which express IE aa 1 to 1487, 323 to 1487, 1 to 960, and 407 to 757 as GST fusion proteins, respectively, were discussed previously (Jang et al., 2001; Kim et al., 1995).

The mammalian expression plasmids pSVIE and pTriEx-TFIIB (1–316) that encode the full-size IE protein and the full-size TFIIB, respectively, were described previously (Smith et al., 1992; Jang et al., 2001). The plasmid pTriEx-TFIIBΔ125–174 was generated by subcloning a *Bst*EII/*Bgl*II fragment of pGST-IIBΔ125–174 into pTriExTFIIB (1–316) digested with *Bst*EII and *Bgl*II. pTriExTFIIBΔ1–123 was created by PCR amplification of the TFIIB sequence that spanned codons 124 to 311. The forward primer was 5′-CCATCGATATGGCAGACAGAATCAATCTAC-3′ and the reverse primer was 5′-CCCAAGCTTTTATAGCT-GTGGTAGTTTGTC-3′. The resulting PCR product was digested with *Hin*dIII and then ligated with *Sma*I/*Hin*dIII-digested pGST-Kg.

### *In vitro transcription/translation*

pG3IE and pGEM44, which express IE (1–1487) and IE (323–1487; IR2 protein), respectively, from the Sp6 promoter in IVTT reactions employing the $T_NT$ coupled rabbit reticulocyte lysate system (Promega) were described previously (Harty and O'Callaghon, 1991; Jang et al., 2001). Plasmid pG3TFIIB, which expresses the entire TFIIB gene in IVTT reactions, was described earlier (Jang et al., 2001). Plasmid G3TFIIBΔ125–174 was expressed in IVTT reactions to generate TFIIBΔ125–174. Plasmid G3TFIIBΔ125–174 was generated by cloning the *Bst*EII–*Bgl*II fragment of pGST-TFIIBΔ125–174 into the *Bst*EII-*Bgl*II sites of pG3TFIIB (1–316).

### *Purification of GST-fusion proteins*

The GST-TFIIB expression plasmids (Fig. 2A) were transformed into *Escherichia coli* (BL21DE3LysE) cells. GST fusion protein synthesis was induced by culturing the transformed bacteria for 1.5 to 2 h at 37°C in 2× YT medium supplemented with 0.5 mM isopropyl-β-D-thiogalactoside (IPTG) as described previously (Jang et al., 2001; Kim et al., 1995, 1997). The soluble GST fusion proteins were purified with the GST Bind Kit as described earlier (Novagen, Madison, WI; Jang et al., 2001). Briefly, bacteria were lysed in the BugBuster Protein Extraction Reagent; the insoluble debris was removed by centrifugation, and the soluble GST fusion proteins were batch-purified using the GST-Bind Resin. The eluted GST fusion proteins were then filtered through Centricon columns (Amicon, Millipore, Bedford, MA) as directed by the manufacturer to both desalt and concentrate the purified proteins. The eluted proteins were next separated by SDS–PAGE and visualized by staining the gel with Coomassie blue (Fig. 2B). The quantity of each fusion protein was determined with the Gel Doc 1000/2000 gel documentation system (Bio-Rad, Hercules, CA) by densitometric comparison of each fusion protein with known amounts of bovine serum albumin. GST-free TFIIB

was generated by incubating 1 μg of GST-TFIIB fusion protein with 1 cleavage unit of thrombin (Sigma Chemical, St. Louis, MO) for 4 h at room temperature. The GST moiety was then removed from the TFIIB preparation by incubating the reaction with GST Bind Resin for 1 h at room temperature.

*In vitro interaction assays*

Two micrograms of each GST-TFIIB protein was incubated with [$^{35}$S]methionine-labeled IE protein or IR2 protein for 1 h at room temperature in binding buffer (100 mM NaCl, 1 mM EDTA, 20 mM Tris-HCl [pH 8.0], and 0.5% NP-40). The GST fusion protein complexes were then precipitated with a 50% slurry of glutathione-Sepharose beads (Pharmacia, Piscataway, N.J.) for 1 h at room temperature. After washing the resin five times with binding buffer, the presence of precipitated $^{35}$S-IE protein or $^{35}$S-IR2 protein in each sample was assessed by SDS–PAGE and autoradiography (Jang et al., 2001). For protein-binding assays involving purified TFIIB or EHV-1-infected cell extracts, the identities of the precipitated proteins were assessed by SDS–PAGE and Western blot analyses (Jang et al., 2001).

*Western blot analysis and antibodies*

Precipitated proteins were resolved in SDS–PAGE gels and electrotransferred to a nitrocellulose membrane (Bio-Rad) for 1 h. After transfer of the proteins, the membrane was blocked for 30 min at RT in TBST buffer containing 5% nonfat powdered milk. The membranes were then incubated for 1 h at RT with either anti-TFIIB rabbit polyclonal antibody (SI-1, Santa Cruz Biotechnology, Santa Cruz, CA) or anti-IE polypeptide antibody (Jang et al., 2001; Smith et al., 1994). The antibodies were diluted 1:1000 in TBST buffer (10 mM Tris–HCl [pH 8.0], 150 mM NaCl, 0.05% Tween 20). The membrane was then washed three times consecutively for 10 min per wash with TBST before incubating the membrane with alkaline phosphatase conjugated goat anti-rabbit antibody (Sigma) diluted 1:10,000 in TBST for 1 h at RT. The membrane was washed once with TBST and subsequently washed twice with AP buffer (100 mM Tris–HCl [pH 9.5], 100 mM NaCl, and 5 mM $MgCl_2$). Finally, the precipitated TFIIB was visualized by incubating the membrane in AP buffer supplemented with 0.165 mg of 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt (BCIP) per milliliter and 0.3 mg of nitroblue tetrazolium chloride (NBT) per milliliter (Invitrogen, Carlsbad, CA).

*Chloramphenicol acetyltransferase (CAT) assays*

Methods for CAT assays that employed the EHV-1 EICP27 promoter-CAT chimera reporter (pEICP27-CAT) have been described elsewhere (Smith et al., 1992; Zhao et al., 1995). The Gal4 two-plasmid assay as well as the expression vector for Gal4-VP16TAD and the Gal4 promoter-CAT reporter construct (pG5EC) were described previously (Smith et al., 1994). Murine fibroblast L-M cells were cultured as monolayers in Eagle's minimum essential medium (EMEM) supplemented with penicillin (100 μg/ml), streptomycin (100 μg/ml), nonessential amino acids, and 5% fetal bovine serum (FBS) (Jang et al., 2001; Smith et al., 1992).

L-M fibroblast cells cultured on 60-mm tissue culture dishes ($2 \times 10^6$ cells per dish) were transfected with plasmid DNA–liposome complexes generated with lipofectin (Invitrogen). Cells were transfected with 1.4 pmol of the pEICP27-CAT reporter. The constructs pSVIE, pTriEx TFIIB (1–316), pTriExTFIIBΔ125–174, pEICP27-CAT, pGal4-VP16TAD, and pG5EC were transfected in molar amounts as indicated in the figure legends. For each transfection, the total amount of DNA transfected per triplicate of plates was adjusted to 20 μg by addition of pUC19 DNA. At 48 to 50 h after transfection, the cells were harvested, and total cell lysates were prepared essentially as described (Bowles et al., 1997, 2000; Kim et al., 1997, 1999; Smith et al., 1992). Each CAT assay was independently repeated at least three times, and within individual experiments, each sample was assayed in triplicate. The data were analyzed for statistical significance by the Student's *t* test.

TFIIBΔ125–174 was compared to TFIIB (1–316) for adverse effects on the steady-state levels of the IE protein by Western blot analyses. L-M fibroblast cells cultured on 60-mm tissue culture dishes ($2 \times 10^6$ cells per dish) were transfected with 0.3 pmol of pSVIE and 0.4 pmol of either pTriEx-1, pTriExTFIIB (1–316), or pTriExTFIIBΔ125–174. Forty-eight hours posttransfection, the cells were lysed in lysis buffer [250 mM Tris–HCl [pH 8.0], 50% M-Per (Pierce, Rockford, IL)], and 40 μg of cell lysate was processed for Western blot analysis with the anti-IE polypeptide antibody as described above.

## Acknowledgments

We thank Suzanne Zavecz for excellent technical assistance and Dr. Paul D. Ebner for helpful comments. This investigation was supported by research Grant AI-22001 from the National Institutes of Health.

## References

Agostini, I., Navarro, J.M., Rey, F., Bouhamdan, M., Spire, B., Vigne, R., Sire, J., 1996. The human immunodeficiency virus type 1 Vpr transactivator: cooperation with promoter-bound activator domains and binding to TFIIB. J. Mol. Biol. 261, 599–606.

Bagby, S., Sungjoon, K., Maldonado, E., Tong, K.I., Reinberg, D., Ikura, M., 1995. Solution structure of the C-terminal core domain of human TFIIB: similarity to cyclin A and interaction with TATA-binding protein. Cell 82, 857–867.

Bangur, C.S., Faitar, S.L., Folster, J.P., Ponticelli, A.S., 1999. An interaction between the N-terminal region and the core domain of yeast TFIIB

promotes the formation of TATA-binding protein-TFIIB-DNA complexes. J. Biol. Chem. 274, 23203–23209.
Bangur, C.S., Pardee, T.S., Ponticelli, A.S., 1997. Mutational analysis of the D1/E1 core helices and the conserved N-terminal region of yeast transcription factor IIB (TFIIB): identification of an N-terminal mutant that stabilizes TATA-binding protein-TFIIB-DNA complexes. Mol. Cell Biol. 17, 6784–6793.
Benson, J.D., Lawande, R., Howley, P.M., 1997. Conserved interaction of the papillomavirus E2 transcriptional activator proteins with human and yeast TFIIB proteins. J. Virol. 71, 8041–8047.
Bowles, D.E., Holden, V.R., Zhao, Y., O'Callaghan, D.J., 1997. The ICP0 protein of equine herpes virus 1 is an early protein that independently transactivates expression of all classes of viral promoters. J. Virol. 71, 4904–4914.
Bowles, D.E., Kim, S.K., O'Callaghan, D.J., 2000. Characterization of the trans-activation properties of equine herpesvirus 1 EICP0 protein. J. Virol. 74, 1200–1208.
Buczynski, K.A., Kim, S.K., O'Callaghan, D.J., 1999. Characterization of the transactivation domain of the equine herpesvirus type 1 immediate-early protein. Virus Res. 65, 131–140.
Buratowski, S., Zhou, H., 1993. Functional domains of transcription factor TFIIB. Proc. Natl. Acad. Sci. USA 90, 5633–5637.
Bushnell, D.A., Bamdad, C., Kornberg, R.D., 1996. A minimal set of RNA polymerase II transcription protein interactions. J. Biol. Chem. 271, 20170–20174.
Carrozza, M.J., DeLuca, N.A., 1996. Interaction of the viral activator protein ICP4 with TEIID through TAF250. Mol. Cell Biol. 16, 3085–3093.
Caughman, G.B., Robertson, A.T., Gray, W.L., Sullivan, D.C., O'Callaghan, D.J., 1988. Characterization of equine herpesvirus type 1 immediate-early proteins. Virology 163, 563–571.
Caughman, G.B., Staczek, J., O'Callaghan, D.J., 1985. Equine herpesvirus type 1 infected cell polypeptides: evidence for immediate-early/early/late regulation of viral gene expression. Virology 145, 49–61.
Chou, S., Struhl, K., 1997. Transcriptional activation by TFIIB mutants that are severely impaired in interaction with promoter DNA and acidic activation domains. Mol. Cell Biol. 17, 6794–6802.
Clements, J.B., Watson, R.J., Wilkie, N.M., 1977. Temporal regulation of herpes simplex virus type 1 transcription: location of transcripts on the viral genome. Cell 12, 275–285.
Derbigny, W.A., Kim, S.K., Caughman, G.B., O'Callaghan, D.J., 2000. The EICP22 protein of equine herpesvirus 1 physically interacts with the immediate-early protein and with itself to form dimers and higher-order complexes. J. Virol. 74, 1425–1435.
Derbigny, W.A., Kim, S.K., Jang, H.K., O'Callaghan, D.J., 2002. EHV-1 EICP22 protein sequences that mediate its physical interaction with the immediate early protein are not sufficient to enhance the trans-activation activity of the IE protein. Virus Res. 84, 1–15.
Frampton, A.R., Smith, P.M., Zhang, Y., Matsumura, T., Osterrieder, N., O'Callaghan, D.J., 2002. Contribution of gene products encoded within the unique short segment of equine herpesvirus 1 to virulence in a murine model. Virus Res. 90, 287–301.
Garko-Buczynski, K.A., Smith, R.H., Kim, S.K., O'Callaghan, D.J., 1998. Complementation of a replication-defective mutant of equine herpesvirus type 1 by a cell line expressing the immediate-early protein. Virology 248, 83–94.
Gonzalez-Couto, E., Klages, N., Strubin, M., 1997. Synergistic and promoter-selective activation of transcription by recruitment of transcription factors TFIID and TFIIB. Proc. Natl. Acad. Sci. USA. 94, 8036–8041.
Gray, W.L., Bauman, R.P., Robertson, A.T., Caughman, G.B., O'Callaghan, D.J., Staczek, J., 1987a. Regulation of equine herpesvirus type 1 gene expression: characterization of immediate-early, early, and late transcription. Virology 158, 79–87.
Gray, W.L., Bauman, R.P., Robertson, A.T., O'Callaghan, D.J., Staczek, J., 1987b. Characterization and mapping of equine herpesvirus type 1 immediate-early, early, and late transcripts. Virus Res. 8, 233–244.
Grondin, B., DeLuca, N., 2000. Herpes simplex virus type 1 ICP4 promotes transcription preinitiation complex formation by enhancing the binding of TFIID to DNA. J. Virol. 74, 11504–11510.
Grundy, F.J., Baumann, R.P., O'Callaghan, D.J., 1989. DNA sequence and comparative analysis of the equine herpesvirus type 1 immediate-early gene. Virology 172, 223–236.
Gu, B., DeLuca, N., 1994. Requirements for activation of the herpes simplex virus glycoprotein C promoter in vitro by the viral regulatory protein ICP4. J. Virol. 68, 7953–7965.
Ha, I., Roberts, S., Maldonado, E., Sun, Z., Kim, L., Green, M., Reinberg, D., 1993. Multiple functional domains of human transcription factor IIB: distinct interactions with two general transcription factors and RNA polymerase II. Genes Dev. 7, 1021–1032.
Hall, D.B., Struhl, K., 2002. The VP16 activation domain interacts with multiple transcriptional components as determined by protein-protein cross-linking in vivo. J. Biol. Chem. 277, 46043–46050.
Hampsey, M., 1998. Molecular genetics of the RNA polymerase II general transcriptional machinery. Microbiol. Mol. Biol. Rev. 62, 456–503.
Harty, R.N., Colle, C.F., O'Callaghan, D.J., 1990. Equine herpesvirus type 1 gene regulation: characterization of transcription from the immediate early gene region in productive infection, in: Wagner, E.K. (Ed.), Herpesvirus transcription and its regulation, CRC Press, Inc., Boca Raton, FL, pp. 319–338.
Harty, R.N., O'Callaghan, D.J., 1991. An early gene maps within and is 3′ coterminal with the immediate-early gene of equine herpesvirus 1. J. Virol. 65, 3829–3838.
Haviv, I., Shamay, M., Doitsh, G., Shaul, Y., 1998. Hepatitis B virus pX targets TFIIB in transcription coactivation. Mol. Cell. Biol. 18, 1562–1569.
Hawkes, N.A., Roberts, S.G., 1999. The role of human TFIIB in transcription start site selection in vitro and in vivo. J. Biol. Chem. 274, 14337–14343.
Holden, V.R., Zhao, Y., Thompson, Y., Caughman, G.B., Smith, R.H., O'Callaghan, D.J., 1995. Characterization of the regulatory function of the ICP22 protein of equine herpesvirus type 1. Virology 210, 273–282.
Hones, R.W., Roizman, B., 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. 14, 8–19.
Ingles, C.J., Shales, M., Cress, W.D., Triezenberg, S.J., Greenblatt, J., 1991. Reduced binding of TFIID to transcriptionally compromised mutants of VP16. Nature 351, 588–590.
Jang, H.K., Albrecht, R.A., Buczynski, K.A., Kim, S.K., Derbigny, W.A., O'Callaghan, D.J., 2001. Mapping the sequences that mediate an interaction between the equine herpesvirus type 1 immediate-early protein and human TFIIB. J. Virol. 75, 10219–10230.
Kim, S.K., Bowles, D.E., O'Callaghan, D.J., 1999. The γ2 late glycoprotein K promoter of equine herpesvirus 1 is differentially regulated by the IE and EICP0 proteins. Virology 256, 173–179.
Kim, S.K., Buczynski, K.A., Caughman, G.B., O'Callaghan, D.J., 2001. The equine herpesvirus 1 immediate-early protein interacts with EAP, a nucleolar-ribosomal protein. Virology 279, 173–184.
Kim, S.K., Holden, V.R., O'Callaghan, D.J., 1997. The ICP22 protein of equine herpesvirus 1 cooperates with the IE protein to regulate viral gene expression. J. Virol. 71, 1004–1012.
Kim, S.K., Jang, H.K., Albrecht, R.A., Derbigny, W.A., Zhang, Y., O'Callaghan, D.J., 2003. Interaction of the equine herpesvirus 1 EICP0 protein with the immediate-early (IE) protein, TFIIB, and TBP may mediate the antagonism between the IE and EICP0 proteins. J. Virology 77, 2675–2685.
Kim, S.K., O'Callaghan, D.J., 2001. Molecular characterizations of the equine herpesvirus 1 ETIF promoter region and translation initiation site. Virology 286, 237–247.
Kim, S.K., Smith, R.H., O'Callaghan, D.J., 1995. Characterization of DNA binding properties of the immediate-early gene product of equine herpesvirus type 1. Virology 213, 46–56.
Kim, Y.J., Bjorklund, S., Li, Y., Sayre, M.H., Kornberg, R.D., 1994. A multiprotein mediator of transcriptional activation and its interaction

with the C-terminal repeat domain of RNA polymerase II. Cell 77, 599–608.
Klemm, R.D., Goodrich, J.A., Zhou, S., Tjian, R., 1995. Molecular cloning and expression of the 32-kDa subunit of human TFIID reveals interactions with VP16 and TFIIB that mediate transcriptional activation. Proc. Natl. Acad. Sci. USA 92, 5788–5792.
Kobayashi, N., Boyer, T.G., Berk, A.J., 1995. A class of activation domains interacts directly with TFIIA and stimulates TFIIA-TFIID-promoter complex assembly. Mol. Cell Biol. 15, 6465–6473.
Koleske, A.J., Young, R.A., 1994. An RNA polymerase II holoenzyme responsive to activators. Nature 368, 466–469.
Koleske, A.J., Young, R.A., 1995. The RNA polymerase II holoenzyme and its implications for gene regulation. Trends Biochem. Sci. 20, 113–116.
Lagrange, T., Kapanidis, A.N., Tang, H., Reinberg, D., Ebright, R.H., 1998. New core promoter element in RNA polymerase II-dependent transcription: sequence-specific DNA binding by transcription factor IIB. Genes Dev. 12, 34–44.
Lewis, J.B., Thompson, Y.G., Caughman, G.B., 1993. Transcriptional control of the equine herpesvirus 1 immediate early gene. Virology 197, 788–792.
Lewis, J.B., Thompson, Y.G., Feng, X., Holden, V.R., O'Callaghan, D.J., Caughman, G.B., 1997. Structural and antigenic identification of the ORF12 protein ($\alpha$TIF) of the equine herpesvirus 1. Virology 230, 369–375.
Lin, Y.S., Ha, I., Maldonado, E., Reinberg, D., Green, M.R., 1991. Binding of general transcription factor TFIIB to an acidic activating region. Nature 353, 569–571.
Myer, V.E., Young, R.A., 1998. RNA polymerase II holoenzymes and subcomplexes. J. Biol. Chem. 273, 27757–27760.
Nikolov, D.B., Chen, H., Halay, E.D., Usheva, A.A., Hisatake, K., Lee, D.K., Roeder, R.G., Burley, S.K., 1995. Crystal structure of a TFIIB-TBP-TATA-element ternary complex. Nature 377, 119–128.
O'Callaghan, D.J., Osterrieder, N., 1999. Equine herpesviruses, in: Webster, R.G., Granoff, A. (Eds.), Encyclopedia of Virology, second ed. Academic Press, Ltd., London, pp. 508–515.
Ossipow, V., Tassan, J.P., Nigg, E.A., Schibler, U., 1995. A mammalian RNA polymerase II holoenzyme containing all components required for promoter-specific transcription initiation. Cell 83, 137–146.
Pardee, T.S., Bangur, C.S., Ponticelli, A.S., 1998. The N-terminal region of yeast TFIIB contains two adjacent functional domains involved in stable RNA polymerase II binding and transcription start site selection. J. Biol. Chem. 273, 17859–17864.
Pinto, I., Wu, W.H., Na, J.G., Hampsey, M., 1994. Characterization of sua7 mutations defines a domain of TFIIB involved in transcription start site selection in yeast. J. Biol. Chem. 269, 30569–30573.
Ranish, J.A., Yudkovsky, N., Hahn, S., 1999. Intermediates in formation and activity of the RNA polymerase II preinitiation complex: holoenzyme recruitment and a postrecruitment role for the TATA box and TFIIB. Genes Dev. 13, 49–63.
Rank, N.M., Lambert, P.F., 1995. Bovine papillomavirus type 1 E2 transcriptional regulators directly bind two cellular transcription factors, TFIID and TFIIB. J. Virol. 69, 6323–6334.
Roberts, S.G., Ha, I., Maldonado, E., Reinberg, D., Green, M.R., 1993. Interaction between an acidic activator and transcription factor TFIIB is required for transcriptional activation. Nature 363, 741–744.
Roizman, B., Kozak, M., Honess, R.W., Hayward, G., 1975. Regulation of herpesvirus macromolecular synthesis: evidence for multilevel regulation of herpes simplex 1 RNA and protein synthesis. Cold Spring Harb. Symp. Quant. Biol. 39 (Pt2), 687–701.
Smith, R.H., Caughman, G.B., O'Callaghan, D.J., 1992. Characterization of the regulatory functions of the equine herpesvirus 1 immediate-early gene product. J. Virol. 66, 936–945.
Smith, R.H., Holden, V.R., O'Callaghan, D.J., 1995. Nuclear localization and transcriptional activation activities of truncated versions of the immediate-early gene product of equine herpesvirus 1. J. Virol. 69, 3857–3862.
Smith, R.H., Zhao, Y., O'Callaghan, D.J., 1993. The equine herpesvirus 1 (EHV-1) UL3 gene, an ICP27 homolog; is necessary for full activation of gene expression directed by an EHV 1 late promoter. J. Virol. 67, 1105–1109.
Smith, R.H., Zhao, Y., O'Callaghan, D.J., 1994. The equine herpesvirus type 1 immediate-early gene product contains an acidic transcriptional activation domain. Virology 202, 760–770.
Stringer, K.F., Ingles, C.J., Greenblatt, J., 1990. An acidic transcriptional activation domain binds directly and selectively to the TATA-box factor. Nature 345, 783–786.
Thompson, C.M., Koleske, A.J., Shao, D.M., Young, R.A., 1993. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell 73, 1361–1375.
Tong, X., Wang, F., Thut, C.J., Kieff, E., 1995. The Epstein-Barr virus nuclear protein 2 acidic domain can interact with TFIIB, TAF40, and RPA70 but not with TATA-binding protein. J. Virol. 69, 585–588.
Tsai, F.T.F., Sigler, P.B., 2000. Structural basis of preinitiation complex assembly on human pol II promoters. EMBO J. 19, 25–36.
Veschambre, P., Roisin, A., Jalinot, P., 1997. Biochemical and functional interaction of the human immunodeficiency virus type 1 Tat transactivator with the general transcription factor TFIIB. J. Gen. Virol. 78, 2235–2245.
Weinheimer, S.P., McKnight, S.L., 1987. Transcriptional and post-transcriptional controls establish the cascade of herpes simplex virus protein synthesis. J. Mol. Biol. 195, 819–833.
Yamashita, S., Hisatake, K., Kokubo, T., Doi, K., Roeder, R.G., Horikoshi, M., Nakatan, Y., 1993. Transcription factor TFIIB sites important for interaction with promoter-bound TFIID. Science 261, 463–466.
Yao, J., Breiding, D.E., Androphy, E.J., 1998. Functional interaction of the bovine papillomavirus E2 transactivation domain with TFIIB. J. Virol. 72, 1013–1019.
Zhang, Y., Smith, P.M., Frampton, A.R., Osterrieder, N., Jennings, S.R., O'Callaghan, D.J. 2003. Cytokine profiles and long term virus-specific antibodies following immunization of CBA mice with equine herpesvirus 1 and viral glycoprotein D. Viral Immunol. 16, 307–320.
Zhao, Y., Holden, V.R., Harty, R.N., O'Callaghan, D.J., 1992. Identification and transcriptional analyses of the UL3 and UL4 genes of equine herpesvirus 1, homologs of the ICP27 and glycoprotein K genes of herpes simplex virus. J. Virol. 66, 5363–5372.
Zhao, Y., Holden, V.R., Smith, R.H., O'Callaghan, D.J., 1995. Regulatory function of the equine herpesvirus 1 ICP27 gene product. J. Virol. 69, 2786–2793.